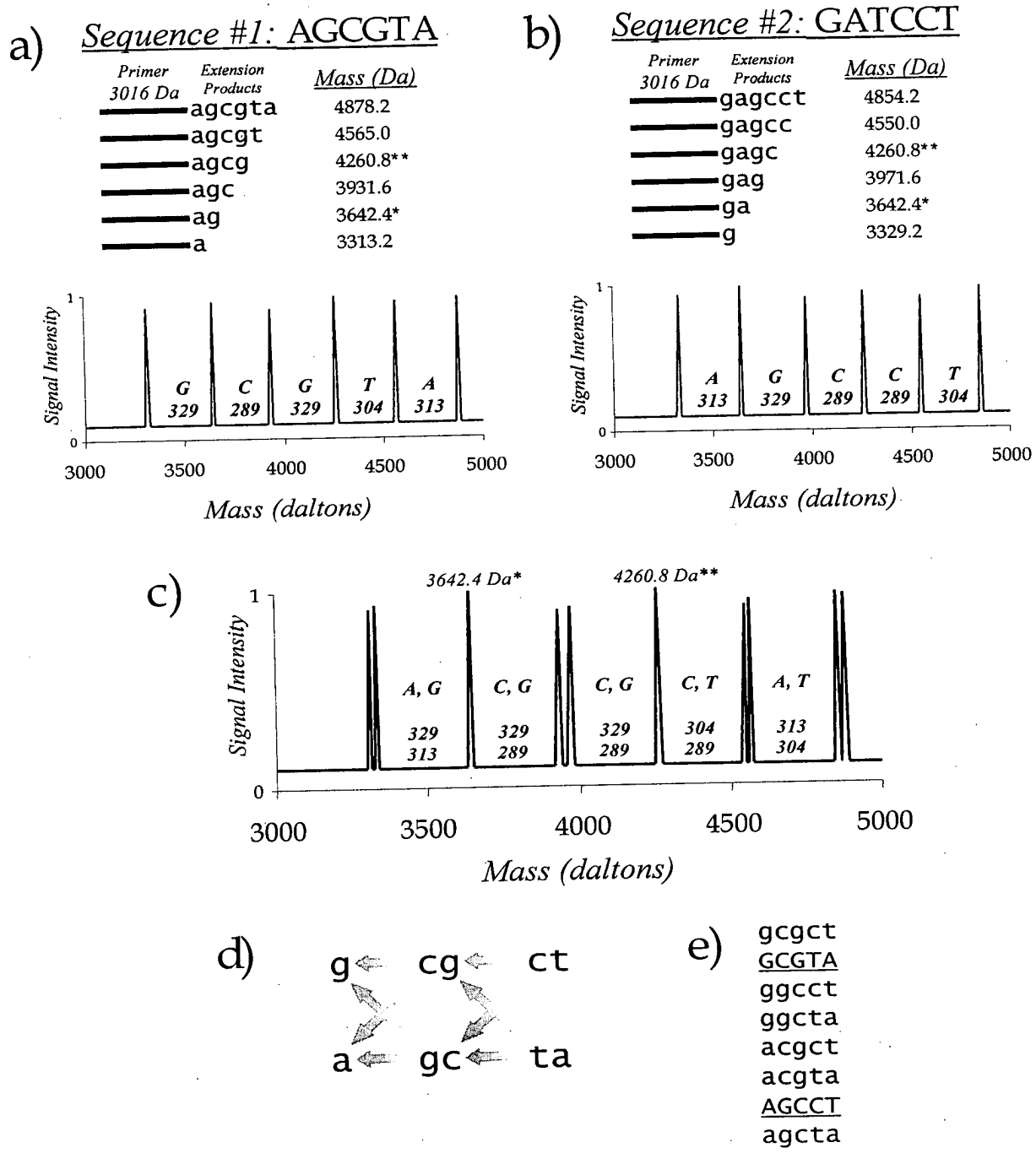

a)
Sequence #1: AGCGTA
Primer 3016 Da
Extension Products
Mass (Da)
agcgta 4878.2
agcgt 4565.0
agcg 4260.8**
agc 3931.6
ag 3642.4*
a 3313.2
Signal Intensity
G 329
C 289
G 329
T 304
A 313
3000 3500 4000 4500 5000
Mass (daltons)
b)
Sequence #2: GATCCT
Primer 3016 Da
Extension Products
Mass (Da)
gagcct 4854.2
gagcc 4550.0
gagc 4260.8**
gag 3971.6
ga 3642.4*
g 3329.2
Signal Intensity
A 313
G 329
C 289
C 289
T 304
3000 3500 4000 4500 5000
Mass (daltons)
c)
3642.4 Da*
4260.8 Da**
Signal Intensity
A, G 329 313
C, G 329 289
C, G 329 289
C, T 304 289
A, T 313 304
3000 3500 4000 4500 5000
Mass (daltons)
d)
g cg ct
a gc ta
e)
gcgct
GCGTA
ggcct
ggcta
acgct
acgta
AGCCT
agcta

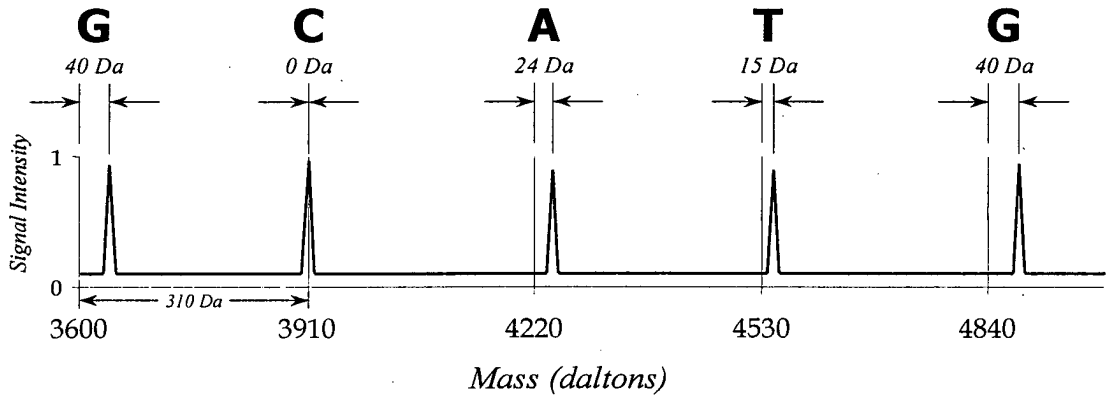
Figure 1

a)

| Nucleotide | Mass (Da) |
|---|---|
| dN | 310 |
| ddC | 273 |
| ddT | 288 |
| ddA | 297 |
| ddG | 313 |

## Sequence #1: GCATG

| Primer #1 3327 Da — Extension Products | Mass (Da) |
|---|---|
| nnnng | 4880 |
| nnnt | 4545 |
| nna | 4244 |
| nc | 3910 |
| g | 3640 |




b)

## Sequence #2: CATGC

| Primer #2 3404 Da — Extension Products | Mass (Da) |
|---|---|
| nnnnc | 4917 |
| nnng | 4647 |
| nnt | 4312 |
| na | 4011 |
| c | 3677 |

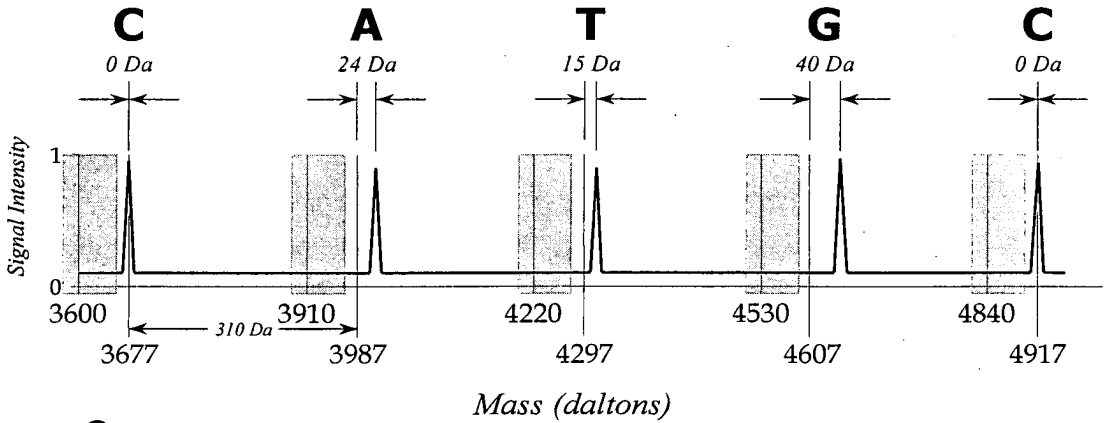



Figure 2

## *Sequence #1:* GCATA

| *Primer #1 3327 Da* | *Extension Products* | *Mass (Da)* |
|---|---|---|
| ——— | nnnna | 4864 |
| ——— | nnnt | 4545 |
| ——— | nna | 4244 |
| ——— | nc | 3910 |
| ——— | g | 3640 |

## *Sequence #3:* CATGC

| *Primer #3 3404 Da* | *Extension Products* | *Mass (Da)* |
|---|---|---|
| ——— | nnnnc | 4917 |
| ——— | nnng | 4647 |
| ——— | nnt | 4312 |
| ——— | na | 4011 |
| ——— | c | 3677 |

## *Sequence #2:* TCAGG

| *Primer #2 3481 Da* | *Extension Products* | *Mass (Da)* |
|---|---|---|
| ——— | nnnng | 5034 |
| ——— | nnng | 4724 |
| ——— | nna | 4398 |
| ——— | nc | 4064 |
| ——— | t | 3769 |

## *Sequence #4:* AACTC

| *Primer #4 3558 Da* | *Extension Products* | *Mass (Da)* |
|---|---|---|
| ——— | nnnnc | 5071 |
| ——— | nnnt | 4776 |
| ——— | nnc | 4451 |
| ——— | na | 4165 |
| ——— | a | 3855 |

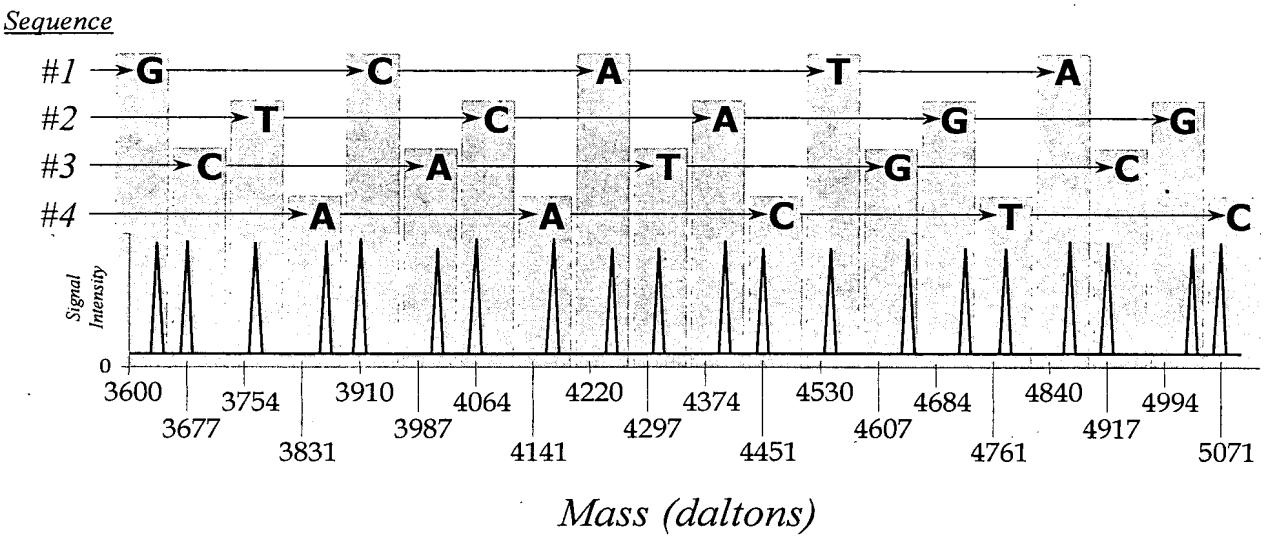



Figure 3

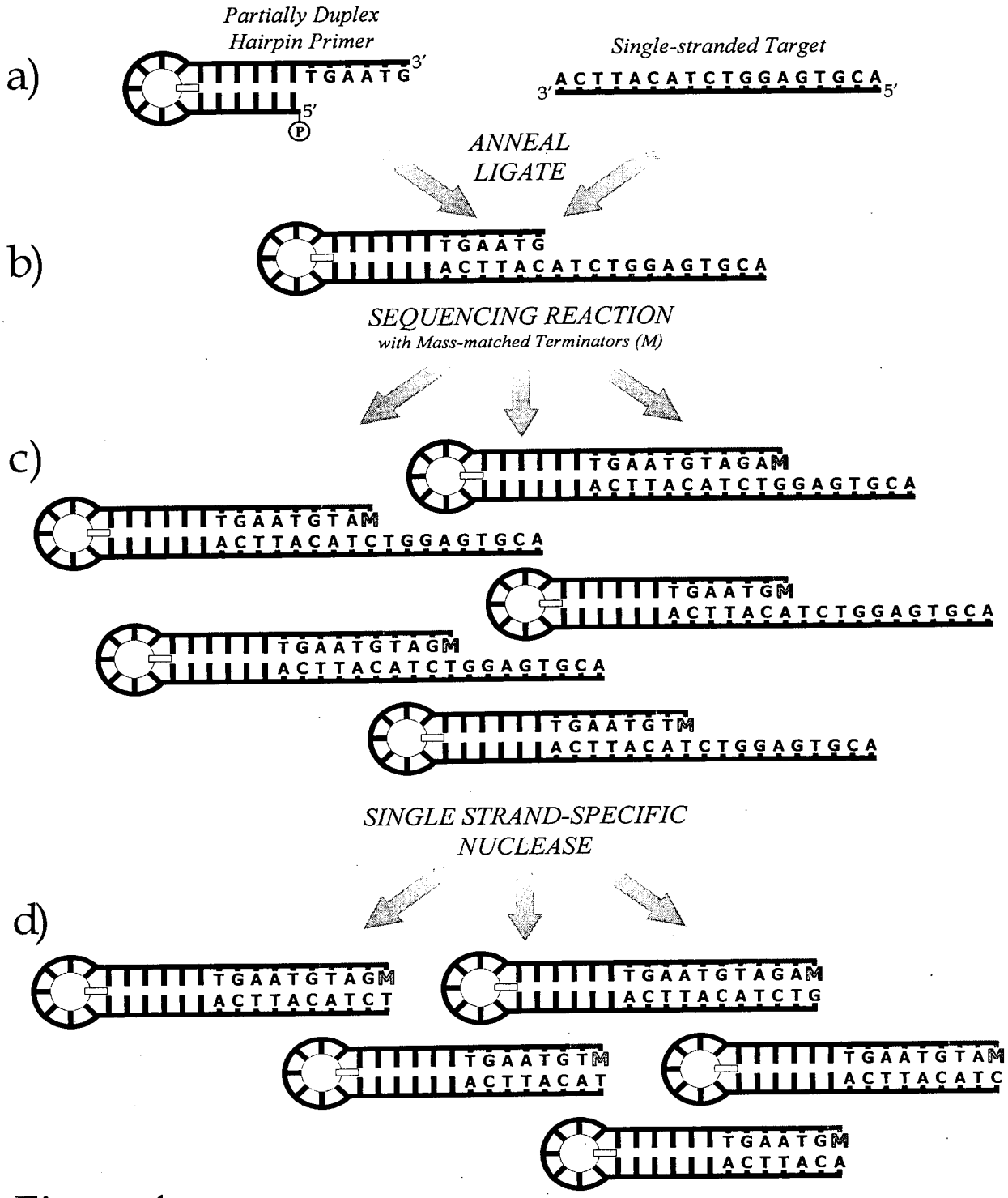
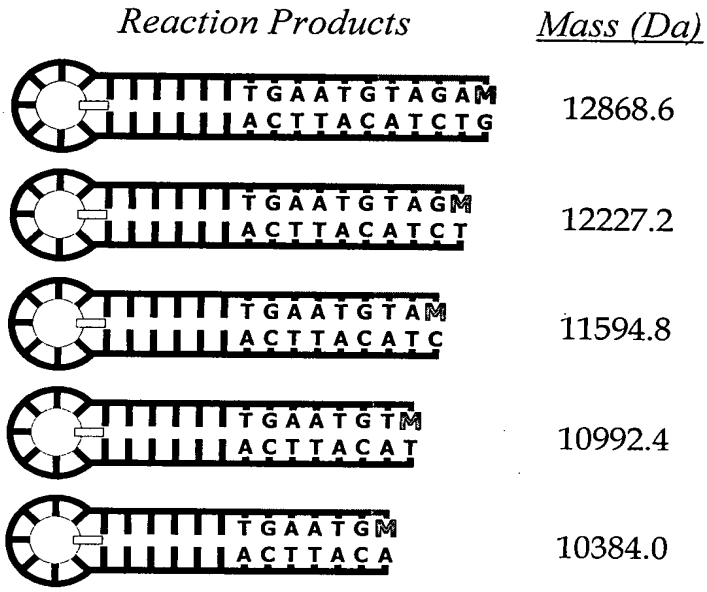

Partially Duplex
Hairpin Primer
Single-stranded Target
a)
TGAATG
3′
5′
P
3′ ACTTACATCTGGAGTGCA 5′
ANNEAL
LIGATE
b)
TGAATG
ACTTACATCTGGAGTGCA
SEQUENCING REACTION
with Mass-matched Terminators (M)
c)
TGAATGTAGAM
ACTTACATCTGGAGTGCA
TGAATGTAM
ACTTACATCTGGAGTGCA
TGAATGM
ACTTACATCTGGAGTGCA
TGAATGTAGM
ACTTACATCTGGAGTGCA
TGAATGTM
ACTTACATCTGGAGTGCA
SINGLE STRAND-SPECIFIC
NUCLEASE
d)
TGAATGTAGM
ACTTACATCT
TGAATGTAGAM
ACTTACATCTG
TGAATGTM
ACTTACAT
TGAATGTAM
ACTTACATC
TGAATGM
ACTTACA

Figure 4

a)


Reaction Products
Mass (Da)
TGAATGTAGAM
ACTTACATCTG
12868.6
TGAATGTAGM
ACTTACATCT
12227.2
TGAATGTAM
ACTTACATC
11594.8
TGAATGTM
ACTTACAT
10992.4
TGAATGM
ACTTACA
10384.0

b)

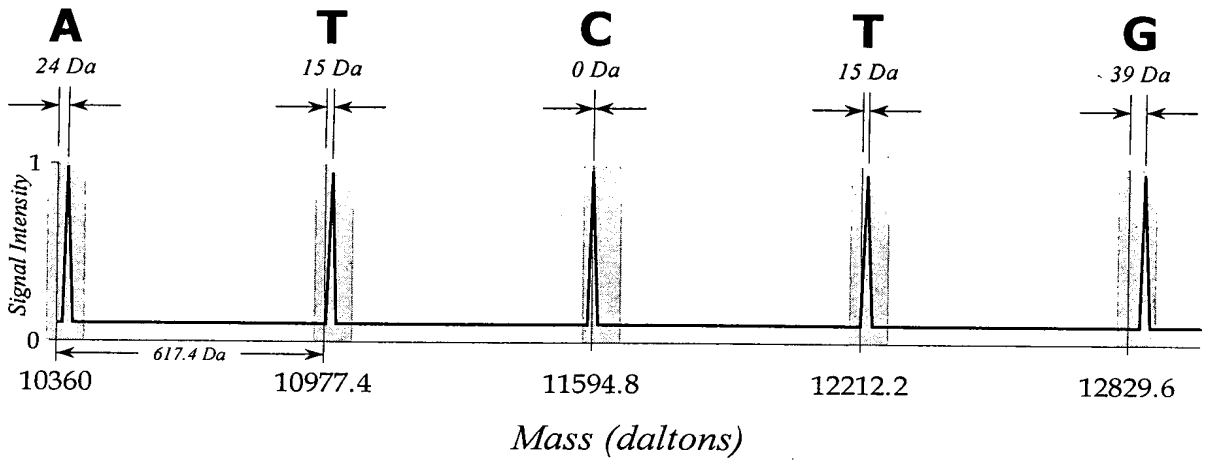

A
24 Da
T
15 Da
C
0 Da
T
15 Da
G
39 Da
Signal Intensity
1
0
617.4 Da
10360
10977.4
11594.8
12212.2
12829.6
Mass (daltons)

Figure 5

a)

*Variant #1:* AACTGCAT

| Primer 3616 Da | Extension Products | Mass (Da) |
|---|---|---|
| | aactgcat | 6054 |
| | aactgca | 5750 |
| | aactgc | 5437 |
| | aactg | 5148 |
| | aact | 4819 |
| | aac | 4515 |
| | aa | 4226 |
| | a | 3913 |

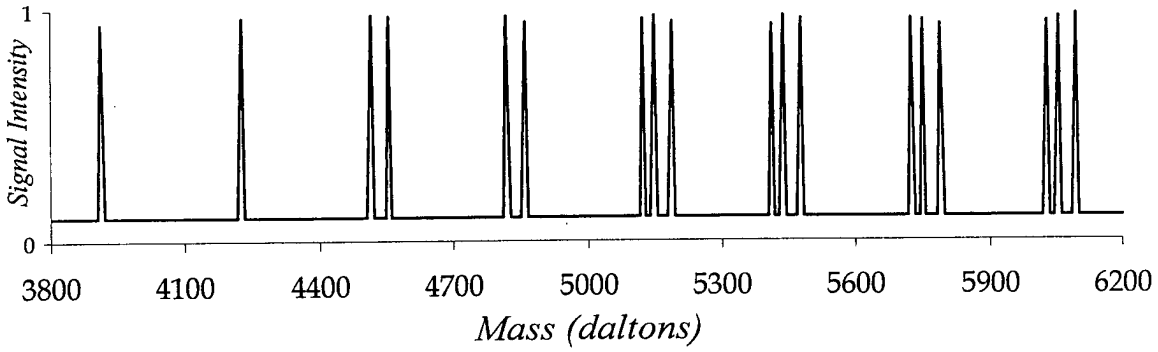
*Variant #2:* AACTTCAT

| Primer 3616 Da | Extension Products | Mass (Da) |
|---|---|---|
| | aactccat | 6029* |
| | aactcca | 5725* |
| | aactcc | 5412* |
| | aactc | 5123* |
| | aact | 4819 |
| | aac | 4515 |
| | aa | 4226 |
| | a | 3913 |

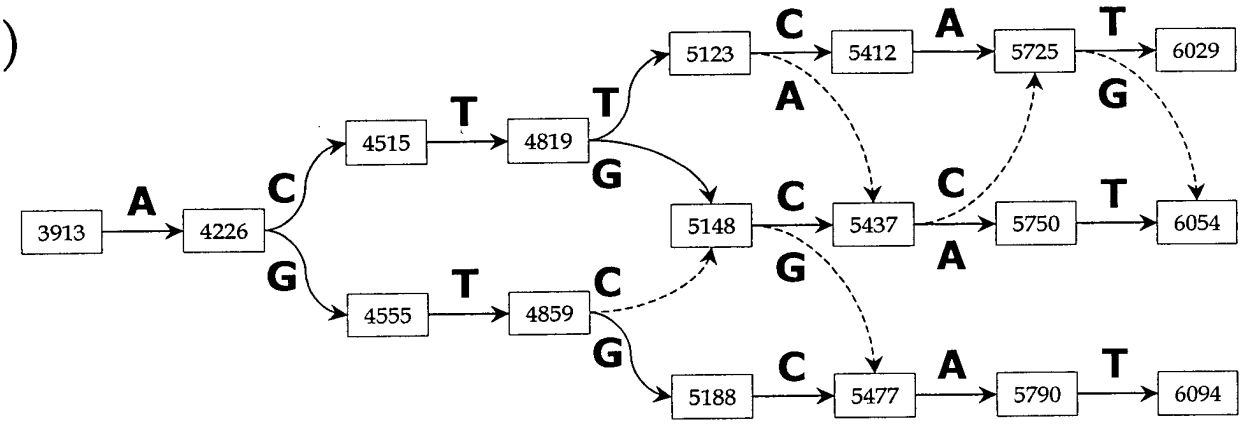
*Variant #3:* AAGTGCAT

| Primer 3616 Da | Extension Products | Mass (Da) |
|---|---|---|
| | aagtgcat | 6094** |
| | aagtgca | 5790** |
| | aagtgc | 5477** |
| | aagtg | 5188** |
| | aagt | 4859** |
| | aag | 4555** |
| | aa | 4226 |
| | a | 3913 |

b)




c)




d)

| | | | | | | |
|---|---|---|---|---|---|---|
| ACTGCAT | actgccg | actgcct | actggat | acttaat | acttacg | acttact |
| acttcag | ACTTCAT | agtccat | agtcccg | agtccct | agtcgat | AGTGCAT |

Figure 6

a)

*Variant #1:* AACTGCAT

| *Primer 3527 Da* | *Extension Products* | *Mass (Da)* |
|---|---|---|
| | nnnnnnnt | 5985 |
| | nnnnnna | 5684 |
| | nnnnnc | 5350 |
| | nnnng | 5080 |
| | nnnt | 4745 |
| | nnc | 4420 |
| | na | 4134 |
| | a | 3824 |

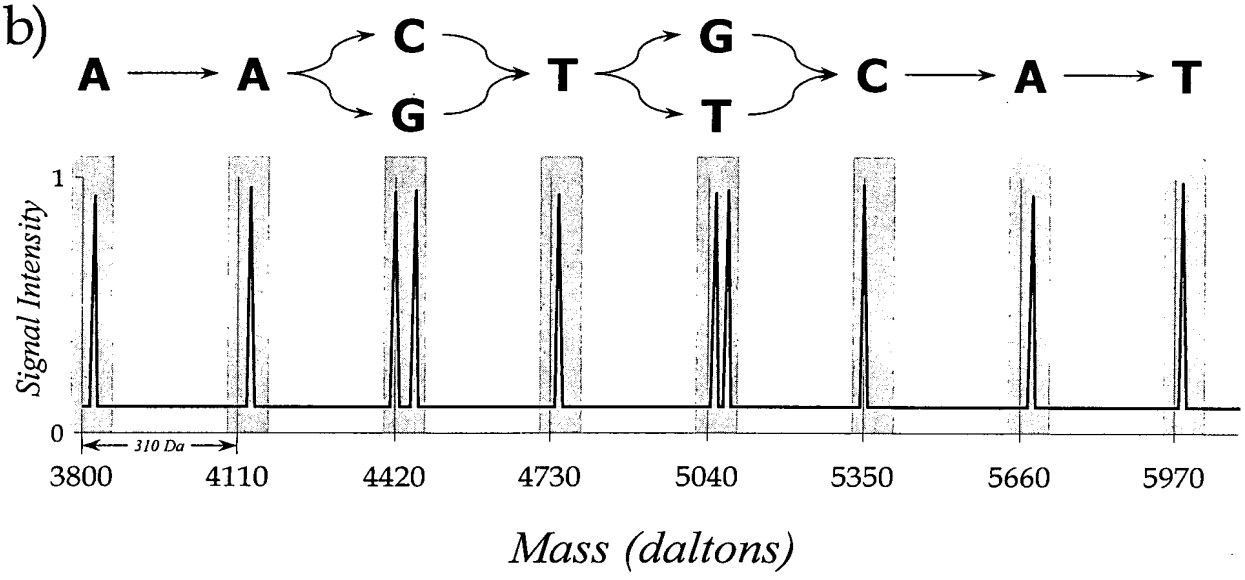
*Variant #2:* AACTTCAT

| *Primer 3527 Da* | *Extension Products* | *Mass (Da)* |
|---|---|---|
| | nnnnnnnt | 5985 |
| | nnnnnna | 5684 |
| | nnnnnc | 5350 |
| | nnnnt | 5055* |
| | nnnt | 4745 |
| | nnc | 4420 |
| | na | 4134 |
| | a | 3824 |

*Variant #3:* AAGTGCAT

| *Primer 3527 Da* | *Extension Products* | *Mass (Da)* |
|---|---|---|
| | nnnnnnnt | 5985 |
| | nnnnnna | 5684 |
| | nnnnnc | 5350 |
| | nnnng | 5080 |
| | nnnt | 4745 |
| | nng | 4460** |
| | na | 4134 |
| | a | 3824 |

b)




c)

$$AA\binom{C}{G}T\binom{G}{T}CAT$$

d)

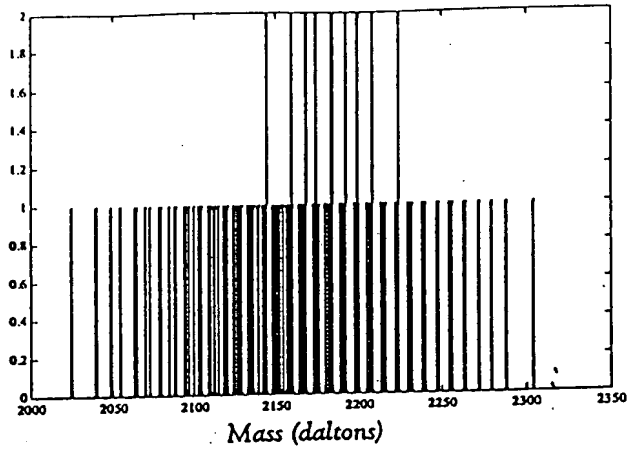
AACTGCAT
AACTTCAT
AAGTGCAT
aagttcat

Figure 7

Figure 8

Base composition density distributions for 7-mers using different nucleotide sets.

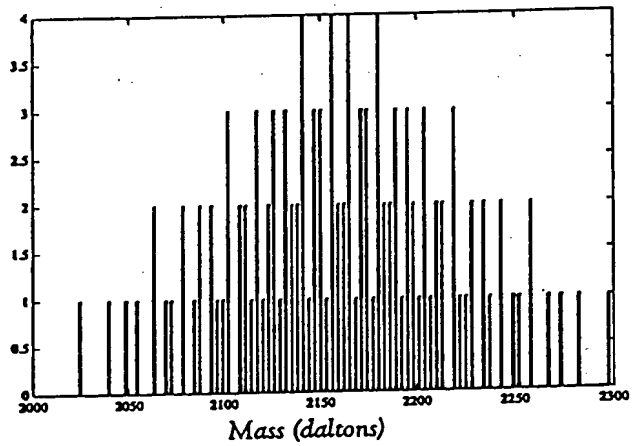
i)




C = 289.2
T = 304.2
A = 313.2
G = 329.2

*Naturally Occurring Bases*
Peaks can be closer than one dalton.
Total No. of different base compositions = 120
Actual number of represented masses = 110
Avg. No. of compositions per mass value = 1.091

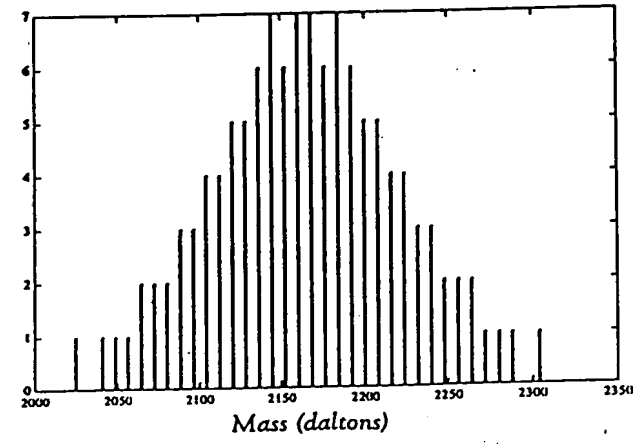
ii)




C = 289.2
T = 304.2
A = 313.2
**G = 328.2**

*Substitution with 7-deaza-dG*
Minimum peak separation = 3 daltons
Number of allowed mass values = 92
Actual number of represented masses = 64
Avg. No. of compositions per mass value = 1.875

iii)




C = 289.2
**T = 305.2**
A = 313.2
G = 329.2

*Substitution with deutero-dT*
Minimum peak separation = 8 daltons
Number of allowed mass values = 36
Actual number of represented masses = 34
Avg. No. of compositions per mass value = 3.529